Histopathological and Some Hematological Analysis in Iraqi Patients with Osteoarthritis

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Abstract

Osteoarthritis (OA) is a biomechanical process in which the joints respond pathologically to mechanical stress, leading to articular cartilage damage and changes in other knee tissue. The present study focuses on some haematological and biochemical test in both patients and control and histopathological change with knee OA patients. Tissue samples (Synovial membrane and Cartilage) obtained from 50 knee OA patients (35 females and 15 males) during total knee replacement for histological study. Blood sample were obtained from 50 knee OA patients and 25 control. The tissue sample and blood were collected during the period from October 2015 to June 2016. In the present study, the Histological study of knee OA tissues (Synovial membrane and Cartilage) expressed many histopathological changes such as fibrin deposition, congestion blood vessels and infiltration of inflammatory cells. Assessment of differential white blood cell (WBC) level in blood revealed that both neutrophils and monocytes levels elevated significantly in total knee OA patients (male and female) in compared with that of healthy controls, but there was no significant difference in neutrophils and monocytes levels of knee OA male patients compared with that of female patients. Also, no significant difference in WBC level of knee OA patients compared with healthy control, but there was a significant difference in WBC level between male and female knee OA patients. On the other hand, no significant difference in lymphocyte level of knee OA patients compared with healthy control for both male and female. Our results showed significant elevation in erythrocyte sedimentation rate (ESR) in females as compared with males with in the patients group. Also there was significant elevation difference in ESR between total patients and controls in males and females. While, no significant difference in platelets level of female with knee OA compared with healthy, but there was highly significant elevation difference in level of platelets in knee OA male compared with control, and between knee OA female and control but there was a significant elevation in platelets level of knee OA patient male as compared with knee OA female. On the other hand, no significant difference in Hemoglobin (Hb) level between male and female knee OA patients as compared with control, and between both males and females.

Keywords: Osteoarthritis, Synovial Membrane, Cartilage, Knee joint.

Introduction

The human knee joint is the biggest and most complex joint in the human musculoskeletal system, which supports the body and facilitates locomotion¹. Osteoarthritis (OA) is the utmost common disorder of the musculoskeletal system, the most common form of arthritis, and the main cause of pain and disability in older adults². OA is a progressive disease which characterized by degeneration of cartilage and alteration of joint tissues leading to pain, stiffness, and disability³. Pain is the most noticeable and disabling symptom of OA, which impacts functions, results in a reduction in activities, and has negative effects on mood, sleep, and quality of life ⁴. The OA can affect all joints in the human body, but the knees, hips, and hands are utmost commonly affected ⁵. Knees OA has the greatest impact on disability and the common parts to be affected are tibiofemoral and lateral patellofemoral parts ⁶.
Physiologically, OA involves alterations in articular cartilage composition, structure and function. Various elements have been referred to as the pathophysiology of OA. Both cartilage degeneration and accelerated bone rotation have been identified as causal elements, though the exact trigger of OA is still not fully understood. It is generally thought that the normal aging increases the sensitivity to OA due to the reduction in the number of chondrocytes in articular cartilage. As a result, cartilage degradation at a faster rate than it is being formed. The current study investigates histopathological changes, some complete blood picture (CBC) and ESR.

Materials and Methods
Control-base study has been designed upon Iraqi patients with OA in the Nursing home hospital and Ghazy Al-hariri hospital for surgical specialties / Medical city and the histopathological study was conducted at Al-Mustansiriya University in the laboratories of Biological Sciences department during the period from October 2015 to June 2016. Subjects involved in this study include 50 patients (35 females and 15 males) at age range 35-80 which diagnosed as OA patient by specialists. Also, 20 normal subjects with gender and age range corresponding to that of patients were involved and considered as a control group.

Sample Collection
Blood samples were collected from all subjects (healthy controls and patients). About 5 ml of blood were aspirated by using peripheral vein punctures and transferred into EDTA tube for some CBC and ESR.

Tissue Collection
The tissue specimens were obtained from 50 OA patients during operations involving total knee replace includes (Synovium and femoral cartilage), then they were kept in the fixative solution (formalin10%) for histopathological study which are routinely stained with Hematoxylin and Eosin (H&E) and Van Gieson’s stain according to Bancroft and Stevens methods.

Results and Discussion
Histopathological Study
A- Synovial Membrane
All histological sections of synovial membrane in knee OA patients with Hematoxylin and Eosin stain showed different changes as presence of numerous new formation of blood vessels, increased synovial stroma also hyperplasia of lining epithelium and inflammatory cells infiltration (Fig.1). Different degrees of inflammatory cells infiltration appearance of lymphocyte cells and macrophage especially around blood vessel, also congestion blood vessel and decrease in diameter by fibrin deposition demonstrated in many histological sections of knee OA patients (Fig.2).
Figure 2: Synovial membrane sections of knee OA patient illustrate A- Inflammatory cells infiltration lymphocyte cells and macrophage especially around blood vessel (H&E, ×10). B- Congestion blood vessel and decrease in diameter (H&E, ×40).

Light microscopy examination of synovial membrane in knee OA patients with Van Giesons' stain demonstrated fibrin deposition, around the synovial villous and many areas filled in by fibrin (Fig. 3).

Figure 3: Synovial membrane section of knee OA patient appearance fibrin deposition, around the synovial villous and many areas filled in by fibrin (orange color) (Van Giesons' staining, x10)

The OA is considered not only a degenerative, but also a proliferative disease. In addition to disruption and loss of the articular cartilage is a hallmark of knee OA, the disease process also results in changes in other knee tissues such as synovial membrane, ligament menisci, subchondral bone.

Synovial membrane histology in classical inflammatory arthritis characterized by a wide heterogeneity. In OA synovial membrane also displays this histological changes but to a lesser degree of inflammation than in Rheumatoid arthritis.

Synovial inflammation will be referred to here as synovitis. Evidence show that inflammation is an integral part of OA progression, Also synovitis seen in OA knees tends to be diffuse and is generally not localized in the areas of chondral defects. Synovitis might occur in the early stage of OA. After cartilage damage the products of cartilage breakdown are released into the synovial fluid. Phagocytic cells in the synovium remove cartilage debris by phagocytosis process which then lead to released pro inflammatory cytokines by synoviocytes causing the synovial membrane to become further inflamed.

Synovitis characterized by hyperplasia of the lining layer, with a dense cellular infiltrate composed largely of monocytes and lymphocytes, through to a synovial membrane which is thickened by fibrotic tissue, blood vessels proliferate, surface fibrin deposition and fibrosis within the synovium.
Our result was in agreement with the result of Benito et al. 14 which observed higher level of macrophage infiltration in the synovium, more blood vessel proliferation and higher markers of vascular proliferation in early OA. Also work by Da et al. 17 reported the infiltration with B cells in the synovium of half OA knees in a group of 41 subjects.

Study of Sakkas and Platsoucas 18 recorded evidence for T-cell activation and the production of Th1 cytokines in OA, with T cells and T-cell infiltrates seen in the synovial membrane. In the other hand Haywood et al. 19 noted the Lymphoid aggregation in the severely inflamed synovial samples.

Femoral Cartilage

Microscopically, the sections of femoral cartilage of knee OA patient using Hematoxylin and Eosin stain showed multiple area of chondrocytes death and sloughing of superficial layer into mid area with disorientation of condones (Fig. 5).

In addition, hypercellularity of superficial layer and disorientation of chondrocyte columns appearance (Fig. 6). On the other hand, all sections demonstrated chondrocyte cluster formation with deposit of hyaline and collagen fibers, many chondrocytes death and loss of matrix in middle zone, abnormal controlling and proliferation of chondrocyte (Fig. 7).

Figure 5: Femoral cartilage section of knee OA patient demonstrate show, A- multiple area of chondrocytes death (arrow). B- Sloughing of superficial layer (arrow) with disorientation of chondrons (head arrow) (H&E, ×10).

Figure 6: Femoral cartilage section of knee OA patient show hypercellularity of superficial layer (arrow) and disorientation of chondrocyte columns (head arrow) (H&E, ×10)
The OA is characterized by the loss of articular cartilage in large synovial joints eventually resulting in the contact of bone on bone. OA related changes in cartilage are sometimes inaccurately considered to be a normal part of the aging process.

The degenerative of knee cartilage may related to chronic inflammation of the Synovium, this inflammation lead to Phagocytosis of cartilage matrix degradation products by the synovial macrophages which then results in the local synthesis of more proteases and pro-inflammatory cytokines, thus proteases diffuse through the synovial fluid to the articular cartilage and induce additional cartilage matrix damage by macromolecular proteolysis, also cytokines stimulate the synthesis of more proteases by chondrocyte.

Age also contributes in cartilage degeneration, with aging, cartilage became less able to resist the forces of repeated mechanical loading and thus became more susceptible to trauma resulting in the early stages of OA. The cartilage damage might be caused by the reduced number of chondrocytes with human aging, which thus fails to regenerate and remodel the cartilage appropriately.

Acute trauma also known to increase the risk for cartilage damage, which could be in the form of a single occurrence or multiple micro traumatic events over a prolonged period.

In addition, chondral lesions involving the knee are common in cartilage injury, injuries to joints also common in humans, especially during sporting activities. The present result was in agreement with the studies of Hwang and Kim and the study of Sandell and Aigner in which that, they reported the proliferation of chondrocytes and exhibit the central feature of chondrocyte death, including hypo cellularity and lacuna emptying in late stage of OA. Also Barbero et al. and Kuhn et al. reported the proliferation of chondrocyte number, the decrease of synthetic and metabolic activity and growth factor, cell death and apoptosis during OA.

**Hematological Study**

Some parameters were investigated in CBC of all healthy and patient subjects: WBC, lymphocyte, monocyte and neutrophil. Changes in concentration of some CBC parameters in different settings and their significance were recorded and shown in (Table 1).
Table 1: Effect of study group and sex in different blood cells of knee OA patient and control

<table>
<thead>
<tr>
<th>Blood cells (10^3/μl)</th>
<th>WBC Mean ± SD</th>
<th>Lymphocyte Mean ± SD</th>
<th>Monocyte Mean ± SD</th>
<th>Neutrophil Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Control</td>
<td>6.52±0.43</td>
<td>7.27±0.30</td>
<td>2.69 ± 0.13</td>
<td>2.53 ± 0.18</td>
</tr>
<tr>
<td>Patients</td>
<td>8.52±0.71*</td>
<td>7.73±0.36**</td>
<td>2.72 ± 0.31</td>
<td>2.45 ± 0.16</td>
</tr>
</tbody>
</table>

*significant difference between patient and control (p < 0.05).
** significant difference between male and female (p < 0.05).
SD: Standard Deviation

In the current study, the WBC level of control male and female were (6.52 ± 0.43 and 7.27 ± 0.30 10^3/μl) respectively. While the WBC level of patient OA male and female were (8.52 ± 0.71 and 7.73 ± 0.36 10^3/μl) respectively.

The results showed non-significant difference (p < 0.05) in WBC level of OA patients compared with healthy control in female. but there is a significant difference in WBC level between male OA patient and male control, and between male and female OA patients. In our study, the significant increase in the concentration of WBC between male and female patients and between normal male and patient even if it exists but remain within normal limits. Study of Hughes et al., 28 showed that there is an increased in the total leucocyte concentration one day after surgery of total hips and total knee replacement. Also Lckinger et al. 29 showed normal WBC count in patient with OA.

The elevation in the level of WBC count might be due to present of accompanies infection and may sever as an early marker for a developing infection and an inflammatory response 30. The levels of lymphocyte for both control and OA patients are shown non-significant difference (p < 0.05) in OA patients compared with healthy control. While monocyte level data show that, the monocyte level in control male is (0.303 ± 0.02 10^3/μl) and for female is (0.377 ± 0.0210^3/μl) while the monocyte level of patient’s OA male is (0.546 ± 0.03 10^3/μl), and for female is (0.554 ± 0.02 10^3/μl).

The results showed a high significant elevation difference in monocyte level of male OA patients compared with that of male healthy control, and between female OA patient and control. There is no significant difference is found between male and female OA patients. Finn 31 reported the increase of monocyte, macrophage cells in 24 to 48 hours in most form of acute inflammation. Also the chronic inflammation primarily mediated by monocyte and macrophage. It is believed that the circulating of high numbers of monocyte allows for rapid migration of large number of cells into damaged tissues in response to inflammatory signals 32. The Information about level of neutrophil show that level of control male is (3.77 ± 0.24 10^3/μl) and for female is (5.62 ± 0.44 10^3/μl) while the neutrophil level of patient’s OA male is (5.62 ± 0.44 10^3/μl), and for female is (5.77 ± 0.17 10^3/μl).

The results showed a significant elevation difference (p < 0.05) in the level of neutrophil of OA patient male compared with that of healthy male, and in the level neutrophil of female with OA compared with that of healthy female.

There is no significant difference neutrophil level of OA patient male compared with that of female Patient. Our study which showed significant increase in neutrophil concentration of patients in compared with control is agreement with Enikő33 which reported the significantly increased in neutrophil concentrations in blood, and synovial fluid of patients with knee OA or meniscus injuries compared to the healthy group. Tasoglu et al.34 considered the neutrophil, lymphocyte ratio levels as inflammatory marker for indicating the severity of knee OA. Neutrophils rapidly respond to chemokine’s, predominate in the inflammatory infiltrate during 6 to 24 hours 31. Also it possibly mediate monocyte/macrophage function35.

After the injury neutrophils, mast cell and macrophages recruited to involved area and can produce variety of pro inflammatory cytokines 36. In contrast with this study Vlad


et al. 37 reported that, OA has been considered a prototypical non-inflammatory arthropathy because of that neutrophils are absent in the synovial fluid, as are systemic manifestations of inflammation.

**ESR Test**

Result in (Table 2) and (Fig. 8) shows that the ESR of control male was (8.35 ± 0.84 mm/hr.) and female was (12.36 ±1.51 mm/hr.) while the ESR of patient with knee OA male was (28.80 ±5.70 mm/hr.) and for female was (36.89 ±3.56 mm/hr.) The results showed a significant elevation (p < 0.05) in ESR of knee OA patient's male and female as compared with control. Also, there is significant elevation difference in ESR of male with knee OA patients when compared with female knee OA patients.

**Hemoglobin (Hb) and Platelets level**

The present result is agreement with the previous study 38,39 which reported that, the elevation in CRP and ESR consider as primary OA inflammation indicator. ESR consider as A non-specific test depending on plasma viscosity, which increases generally in the present of inflammatory response 40.

**Table 2: Effect of study group and sex in ESR level in knee OA patients and control**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Patients</td>
</tr>
<tr>
<td>Male</td>
<td>8.35 ± 0.84</td>
<td>28.80 ±5.70*</td>
</tr>
<tr>
<td>Female</td>
<td>12.36 ±1.51</td>
<td>36.89 ±3.56*</td>
</tr>
</tbody>
</table>

*significant difference between patient and control (p < 0.05).
**significant difference between male and female (p < 0.05).
SD: Standard Deviation. ESR = Erythrocyte Sedimentation Rate

**Figure 8: Effect of study group and sex in ESR**

The (Table 3) and (Fig. 9) show that the Hb level of control male is (12.60 ± 0.31 g/dl) and for female is (13.10 ± 0.40 g/dl) while the Hb level of patient's OA male is (12.31 ± 0.30 g/dl), and for female is (12.64 ± 0.31 g/dl). Statistically no significant (p<0.05) difference is found in Hb level between OA patient as compared with control, and between both male and female OA patients.

**Table 3: Effect of study group and sex in Hemoglobin and Platelets level of knee OA patient and control**

<table>
<thead>
<tr>
<th>Sex g/dl</th>
<th>Hemoglobin Mean ± SD</th>
<th>Platelets Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Patients</td>
</tr>
<tr>
<td>Male</td>
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</tr>
<tr>
<td>Female</td>
<td>13.10 ± 0.40</td>
<td>12.64 ± 0.31</td>
</tr>
<tr>
<td>P-value</td>
<td>...</td>
<td>0.573</td>
</tr>
</tbody>
</table>

*significant difference between patient and control (p < 0.05).
**significant difference between male and female (p < 0.05).
SD: Standard Deviation.
Our study showed that there is no effect of OA disease on Hb level, because there is no change in the concentration in the blood, our result is agreement with study of Walker, et al. which reported normal range of Hb in patient with OA. Data presented in (Table 3) and (Fig.10) show that, the platelet level of control male is ($293.78 \pm 10.07 \times 10^3$ μl) and for female is ($257.18 \pm 17.5 \times 10^3$ μl), while the platelets level of patients with OA male is ($241.46 \pm 14.18 \times 10^3$ μl) and for female OA is ($269.43 \pm 11.55 \times 10^3$ μl). The results showed non-significant difference ($p < 0.05$) in platelets level of female with OA in compared with control. But there is highly significant elevation difference in level of platelets in males with OA compared with control male. Also the results showed a significant elevation in platelets level of OA patient’s male as compared with OA female. Our result showed significant increase in the level of platelets in female patient compared to male patient, these difference may be related to the effect of gender difference on platelet. Differences in the biology of vessel wall between men and women, as well as the direct influence of sex hormones (oestrogens, androgens or progesterone) on platelets or their indirect effect on the vasculature.

**Conclusion**

From the present study can conclude that:
• Histopathological changes in Synovium and Cartilage in knee OA patients occurred with the progression of OA disease.
• The histopathological changes showed infiltration of macrophage and lymphocyte to the side of inflammation, thus reflect the role of these cell in inflammatory response. In addition, increase fibrin deposition in much area of tissues.
• Increase the level of WBC, monocyte and neutrophil in patients with knee O A, this increased might be related to present of inflammatory response.

References


